TOWARD MONODISPERSE POLY(γ–BENZYL α,L-GLUTAMATE): UNIFORM, POLAR, MOLECULAR RODS

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INTRODUCTION

Poly(γ-benzyl α,L-glutamate) (PBLG) has been widely used in studies of the physics of rod-like polymer chains [1]. The helical structure of PBLG gives rise to considerable chain stiffness, such that the persistence length of the chain is on the order of 70 nm in helicogenic solvents [2]. This feature, coupled with the ease of synthesis and good solubility of the polymer has made PBLG the system of choice for the study of both isotropic [2,3] and liquid crystalline [4] solutions of rod-like macromolecules.

The structural properties of PBLG in the solid state and in thin molecular films have also been of interest. Most recently, McMaster and coworkers have published a striking set of scanning tunnelling micrographs (STM) of PBLG deposited from dimethylformamide on highly oriented pyrolytic graphite (HOPG) [5]. Although the authors are appropriately cautious in interpreting their results, they propose that the STM images arise from two-dimensional crystals of PBLG helices aligned in parallel on the HOPG surface. If this interpretation is correct, these micrographs represent the first successful imaging of polypeptide secondary structure by the STM method.

In all of this work, the heterogeneity in molecular weight (i.e., the polydispersity) of the chain population is a complicating factor. For example, Mead and Larson [3] have shown that the effective rotary diffusivity (D_r) for rod-like chains in semi-dilute solutions scales as:

$$D_r \sim \phi^{-2} M^{-7} \ln M$$

where ϕ is the volume fraction of rods and M is the molecular weight. The very strong dependence of D_r on M ensures that the observed rheological and rheo-optical behavior of PBLG will be markedly sensitive to polydispersity.

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It is also likely that the apparent phase behavior of PBLG (and other rod-like macromolecules) is dependent on polydispersity. Horton, Donald and Hill have recently reported the coexistence of two liquid crystalline phases in solutions of PBLG in benzyl alcohol [4], in apparent verification of the predicted "cap" in the phase diagram for rod-like molecules in solution. While we do not dispute this interpretation, the PBLG sample used in this work is of unspecified polydispersity, raising the possibility that the coexisting phases arise from chain-length dependent segregation of the polymer into liquid crystalline domains of differing structure.

The standard synthetic route to PBLG involves the ring-opening polymerization of the N-carboxy-α-amino acid anhydride of benzyl glutamate [1]. Because this method affords heterogeneous populations of chains, many workers have used fractionated samples to reduce ambiguities associated with polydispersity [2]. A direct synthesis of monodisperse PBLG would be welcome.

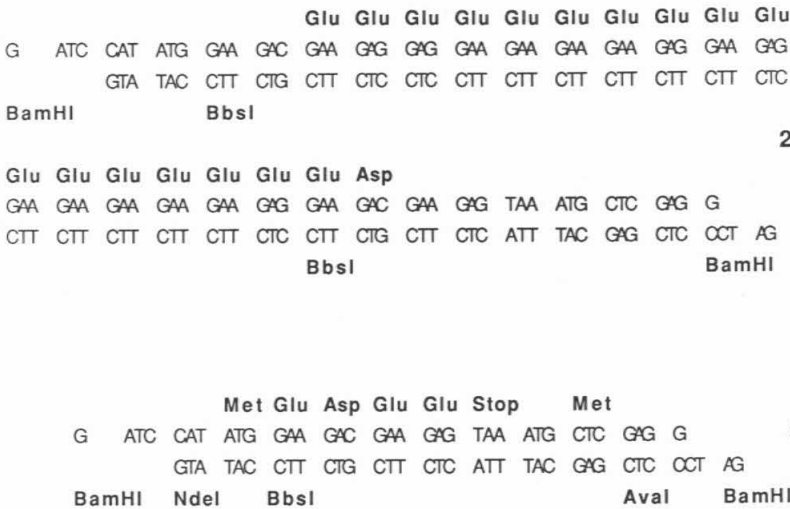
We have recently begun a general exploration of the use of artificial genes to direct the biological synthesis of new polymeric materials [6-8]. As part of this program, we have undertaken the synthesis of monodisperse derivatives of poly(α,L-glutamic acid) (PLGA), logical precursors for monodisperse PBLGs. The details of our synthetic methods have been submitted elsewhere for publication [9]. In the present paper, we discuss the essential features of the synthesis and comment briefly on potential uses for monodisperse PBLGs.

SYNTHESIS

A double-stranded DNA encoding polypeptide sequence **1** was constructed via the following procedure.

H—GluAsp(Glu17Asp)4GluGlu—OH **1**

The oligonucleotide duplex **2** was prepared on a Milligen/Biosearch DNA Synthesizer and cloned and amplified in *E. coli* strain DH5αF'. Digestion of the isolated plasmid DNA with *Bbs*I followed by isolation and self-ligation of the 54 base-pair *Bbs*I fragment afforded the population of multimers with degrees of polymerization up to ca. 20. The ligation product was inserted into the unique *Bbs*I site of a modified pUC18 plasmid bearing the adaptor sequence **3**, and a DH5αF' clone harboring an artificial DNA tetramer was isolated. The *Bam*HI DNA fragment encoding sequence **1** was then transferred to



expression vector pGEX-3X [10]. DH5αF' cells transformed with the recombinant plasmid were grown in YT medium and protein synthesis was induced by addition of isopropyl β-D-thiogalactopyranoside (IPTG). The recombinant protein was purified by affinity chromatography on glutathione-linked Sepharose 4B [11], and sequence **1** was liberated by CNBr digestion. The resulting product was observed to run as a single tight band in polyacrylamide gel electrophoresis under non-denaturing conditions.

Several aspects of this synthetic approach deserve comment. First, polymer **1** is not strictly a PLGA, but instead incorporates four aspartic acid residues spaced at intervals of 18 amino acids. This structure was dictated by the fact that glutamic acid is encoded by only two DNA triplets, GAA and GAG, and by our strategy of cloning the "monomeric" DNA prior to its liberation by enzymatic digestion. Incorporation and subsequence use of *any* enzymatic restriction site into an uninterrupted PLGA coding sequence would require that the remainder of the sequence consist of only a single codon (to avoid multiple cutting of the monomeric DNA). We preferred to avoid long runs of either of the two codons, in order to reduce the risk of genetic instability in the construct. The choice of aspartic acid as a periodic replacement for glutamic acid was based on the chemical similarity of the two amino acids, and on the fact that the Glu-Asp dyad generates a recognition site for *Bbs*I, an efficient, readily available, restriction enzyme. The 18-amino acid spacing of the aspartic acid residues should place all four such residues on the same face of the α-helix (an

185 helix) of the resulting PBLG; the consequences of this kind of substitution are not readily foreseen.

A second point of interest concerns the length of sequence **1.** The 1.5 Å rise per residue characteristic of the α-helix suggests a molecular length of ca. 114 Å for **1.** Given an estimate of ca. 16 Å for the diameter of the PBLG helix [2], the expected axial ratio of the benzyl ester of **1** would be approximately 7. While this is a modest shape anisotropy, the 100 Å chain length is of interest with respect to the fabrication of oriented PBLG surface layers, as described below. It is also worth noting that the "ladder" of multimers obtained on self-ligation of the DNA monomer suggests that the synthetic scheme described herein should readily provide PLGAs of a variety of chain lengths.

Finally, the synthesis of **1** is of limited efficiency, with ca. 4 mg of fusion protein isolated per liter of fermentation medium. Efforts are underway to improve the efficiency of the fermentation, and to develop simple methods for quantitative side-chain benzylation.

FUTURE WORK

As discussed in the Introduction, we intend to use monodisperse PBLGs in the study of the solution and surface properties of rod-like chains. In particular, we are interested in preparing oriented PBLG monolayers on metallic substrates, by taking advantage of uniquely placed cysteine residues to bind either the C-terminus or the N-terminus of the chain at the metallic surface. Similar objectives have been expressed by Samulski and coworkers in this symposium. Should it prove possible to orient monodisperse chains in this fashion, we should be able to control both the thickness and the polarization [12] of the PBLG layer, and thus to fabricate piezoelectric films. Further engineering of the polypeptide chain, by the incorporation of receptor sites at the "top" surface of the film, might then be expected to provide a basis for new sensor technologies. We are currently pursuing these ends.

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